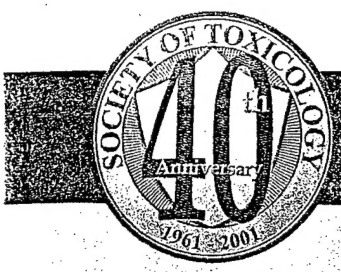

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the organoclay/sand composite was able to reduce the contaminant load of PCP in the oil-water effluent from 83 ppb to 0.22 ppb. Benzo[a]pyrene (BaP) concentration was reduced from 1.5 ppb to 0.0048 ppb. In the bioreactor effluent, the concentrations of PCP and BaP were reduced by 82% and 89%, respectively. Our results demonstrated that this composite was effective in reducing high concentrations of PAHs and PCP. In ongoing field studies, we are assessing the ability of other porous, clay-based composites to remediate creosote contaminated water and to effectively polish bioreactor effluent. Supported by funding from the Texas Agricultural Experiment Station (H6215) and NIH P42-ES04197.

1094 INFLUENCE OF ACUTE ORAL EXPOSURE TO RDX ON NORTHERN BOBWHITE: *COLINUS VIRGINIANUS.*

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RDX (1,3,5-trinitro-1,3,5-triazine) is a highly explosive, solid-form synthetic compound employed for military defense. It has been shown to be present in the soil at many military installations and bioaccumulate in the food web. Studies using rodents have shown RDX exposure to adversely affect the liver and have adverse neurological effects. Numerous studies have shown that some environmental contaminants (*e.g.*, endocrine disruptors, pesticides, and antibiotic residues) can negatively affect avian populations. To date, no studies have been found which have assessed the effects of RDX exposure to birds. The purpose of this study was to evaluate the effects of acute and sub-acute oral exposure of 1,3,5-trinitro-1,3,5-triazine (RDX) to Northern Bobwhite, Colinus virginianus. Adult male and female quail were orally administered 99% pure RDX. Doses were administered by gavage with water as the vehicle. A single oral dose of 187 mg/kg for the female and 280 mg/kg for the male was the estimated Acute Lethal Dose (ALD) after 14 days of observation. Following the ALD, a sub-acute study was initiated in which birds were fed RDX-bound feed *ad libitum* for 14 days. Six groups of 12 birds each (6 male and 6 female) were exposed to 0 mg/kg, 83 mg/kg, 125mg/kg, 187 mg/kg, 280 mg/kg and 420 mg/kg RDX/feed, respectively. The effects of sub-acute feeding included: (a) decreased feed consumption; (b) decreased in weight gain; (c) increased packed cell volume in the peripheral blood of females; (d) declined total plasma protein in females; (e) increased heterophils in the peripheral blood; (f) increased heterophil/lymphocyte ratio in the peripheral blood; and, (g) declined egg production. This is the first study that has examined the effects of RDX exposure in birds and suggests that exposure to RDX may have ecological importance.

1095 ABNORMALITIES IN SNAPPING TURTLES ENVIRONMENTALLY EXPOSED TO PCBS.

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Snapping turtles (Chelydra serpentina) are commonly found in fresh water streams, lakes, and rivers throughout the eastern United States. Previous studies in our laboratory have shown histological abnormalities in the liver, thyroid, and gonads in several species of passerines environmentally exposed to polychlorinated biphenyls (PCBs), as well as asymmetrical patterns in the brain and in paired organs. Snapping turtles were collected from a PCB laden lagoon, a contaminated stream, and an uncontaminated reference site. Morphological features of the turtles were measured, the turtles were sacrificed and necropsied, and the weights of each organ were recorded. Portions of the livers were immediately frozen for biochemical analysis. Pieces of the thyroid, liver, and gonads were embedded in paraffin, sliced into 8-micron sections, stained using Hematoxylin and Eosin, and microscopically analyzed. Image analysis software was used to measure and quantify the endpoints of the abnormalities being investigated. Biochemical analysis showed that glutathione reductase activity was significantly (p=.0146) decreased in specimens from the contaminated sites. EROD (7-ethoxyresorufin 0-deethylase) activity was not significantly different between sites. Comparison of the paired organ weights show that paired organs from the uncontaminated sites were symmetric. Asymmetry was discovered in the gonads from both contaminated sites but other organs did not exhibit this abnormality. At this time, the asymmetry results do not include brain measurements. There were no significant inter-site differences in somatic indices of the major organs. The preliminary results of the histological analysis of the thyroid show visible and detectable differences between contaminated and uncontaminated sites in the size of the colloid area, size of follicles, and the height of epithelial cells.

1096 GLUTATHIONE S-TRANSFERASE EXPRESSION IN HEPATIC LESIONS OF BROWN BULLHEAD (*ameriurus nebulosus*) FROM THE CUYAHOGA RIVER, OHIO.

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The glutathione S-transferases (GSTs) are a multigene family of enzymes important in the detoxification of certain electrophilic carcinogens. Alterations of GST expression in hepatic preneoplastic and neoplastic lesions have been reported in fish exposed to environmental carcinogens, and cellular GST expression may be an important determinant of growth and progression of chemical-associated liver tumors. In the present study, GST expression was examined in hepatic lesions of 44 brown bullheads collected in 1999 from the Cuyahoga River, a highly industrialized site located in Cleveland, Ohio. GST proteins were detected by using a three-step immunohistochemistry method and polyclonal antibodies raised against affinity-purified striped bass liver GST or a channel catfish pi-like GST isoform. Histopathological examination revealed hepatocellular preneoplastic lesions, also known as foci of cellular alteration (FCA), in 34% (15/ 44) of the bullheads examined. Seven of 44 fish examined (15%) exhibited tumors of biliary origin, and a majority of the fish examined exhibited varying degrees of biliary fibrosis and biliary hyperplasia. GST expression in the fibrotic and hyperplastic tissue as well as FCA and tumors were not significantly different from surrounding normal hepatocytes and biliary epithelium. In addition, no differences in hepatic GST expression were observed using the two different fish GST antibodies. Our results indicate that GST expression in hepatic lesions of brown bullhead exposed to environmental carcinogens does not appear to differ significantly from surrounding normal cells. Thus, expression and function of fish hepatic GSTs in neoplastic development following exposure to environmental carcinogens is likely to vary by species. (Supported by NIH P42 ES07375).

1097 EXCITATORY EFFECTS OF NMDA IN FISH BRAIN REGIONS CHARACTERIZED BY THE 2-DEOXYGLUCOSE METHOD.

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Fish are often utilized as indicators of environmental stress, particularly in connection with monitoring changes in water quality. However, neurotoxic endpoints can be insensitive and nonspecific. We have developed a neurochemical method for evaluating environmental neurotoxins and other agents, according to specific regional activities within the CNS. Our system is a unique adaptation of the 2-deoxyglucose (2-DG) method developed by Louis Sokoloff (1977) who demonstrated a direct relationship between glucose metabolism and brain activity at the regional level. Our hypothesis was based on the assumption that changes in neural activity as a result of toxicant exposure would effect the amount of radiolabeled 2-DG accumulated regionally in a fish's brain. Living, healthy fish (Tilapia nilatica, 10g-bw) were injected intramuscularly with 2μCi of 14C 2-DG. Three fish were allowed to swim freely (30min.) in freshwater with added NMDA (20μM) or control (freshwater only). Fish were sacrificed with a lethal dose of MS222 (0.5g/L), whole brains removed, quick-frozen, and stored at −80°C. Brains were cryosectioned (15μm) and sections thaw-mounted onto frost-free microscope slides. Slides were fixed in 10% NBF (20min.) and rinsed with water, then coated with liquid photographic emulsion and exposed for 4 weeks in a light-tight desiccator box, then manually developed. A microscope fitted with both dark and bright field optics allowed visualization of both cytoarchitecture and 2-DG localization within the same field of view. The experiment was repeated in triplicate. Observable increases in regional 2-DG uptake were evident in all NMDA treatment groups compared to controls and specific areas of increased 2-DG uptake included the telencephalon, optic tectum, and regions of the cerebellum, areas with high concentrations of NMDA-subtype glutamate receptors in Tilapia monsanbica (Tong *et al.*, 1992). These results are consistent with the known neural excitatory action of NMDA. Supported by NIEHS PP 1 P01 ES09563.

1098 INHIBITION OF CYP1A-MEDIATED METABOLISM OF BENZO(A)PYRENE (BAP): EFFECTS UPON BAP-INDUCED IMMUNOTOXICITY IN JAPANESE MEDAKA (*ORYZIAS LATIPES*).

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Besides being a potent chemical carcinogen, benzo(a)pyrene (BaP) has also been shown to suppress the immune response of mammals. Furthermore, studies in this laboratory have shown that in vivo exposure of the Japanese medaka (Oryzias

latipes) to BaP results in decreased host immunocompetence. Overall, exposure of both fish and mammals results in decreased numbers of antibody forming cells (AFCs) following immunization with sheep red blood cells (SRBCs). This suppression of humoral immunity is not seen in either species following exposure to the BaP congener, benzo(e)pyrene (BeP). BeP has low affinity for the Aryl hydrocarbon receptor (Ahr) and is not readily metabolized by CYP1A. Previously published studies in rodents have shown that the production of BaP metabolites by CYP1A catalysis may be necessary for resulting immunotoxicity. Thus, for these studies medaka were administered a single IP injection of BaP at either 2, 20, or 200 ug/g BW alone or combined with equimolar amounts of various Ahr / CYP1A antagonists including, alpha-naphthoflavone (ANF), ellipticine, and dehydroepiandrosterone. Forty-eight hr after injection, fish were immunized with SRBCs. A modified Jerne plaque forming cell (PFC) assay, performed 11 days post-immunization, demonstrated that co-exposure of medaka to BaP (20 and 200 ug/g BW) and ANF resulted in AFC numbers similar to those observed in corn oil-injected animals. Thus, BaP in conjunction with ANF ameliorated the suppression of AFC numbers observed following exposure to BaP alone. Given that administration of an Ahr antagonist alleviates BaP-induced immunotoxicity in medaka, the Ahr signaling pathway appears to be necessary for the observed alterations in humoral immunity produced by BaP in fish, as appears to be true for mammalian species (DAAG 55-98-1-0218).

1099 EFFECTS OF SALINITY ON ALDICARB TOXICITY ON JUVENILE RAINBOW TROUT (*ONCOUHYNCHUS MYKISS*) AND STRIPED BASS (*MORONE SAXATILLIS*).

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Aldicarb(Temik), a carbamate pesticide, is widely used throughout the world. Due to the high water solubility of aldicarb, estuarine organisms might be at a great risk for exposure to these chemicals during agricultural runoff. Fluctuations in several environmental variables, such as salinity, can influence the interactions between organisms and pollutants and therefore affect the toxicity of xenobotics. In this study, after fish were acclimated to 3 salinity regimens of 1.5, 7 and 21 ppths for 2 weeks followed by exposure to 0.56 ppm aldicarb, 96h mortality and cholinesterase levels were determined. Salinity significantly enhanced aldicarb toxicity and cholinesterase inhibition in rainbow trout but not in striped bass. To examine the possible mechanisms for the salinity-related toxicity, flavin-containing monooxygenases (FMO) mRNA levels and catalytic activities were also measured after exposing the fish to different salinity regimens for 2 weeks. Salinity increased FMO mRNA expression and catalytic activities in rainbow trout, which was well correlated with the salinity-induced enhancement of aldicarb toxicity. But salinity did not alter FMO mRNA expression and catalytic activities in striped bass, which was also consistent with the salinity-independent aldicarb toxicity. These results suggest that salinity-mediated enhancement of aldicarb toxicity is at least partially due to the salinity-related upregulation of FMO(s), which, in turn, increases the bioactivation of aldicarb to aldicarb sulfoxide, which is a more potent inhibitor of cholinesterase than aldicarb.

1100 SUBLETHAL TOXICITY OF FIELD WATER SAMPLES CONTAMINATED WITH ESFENVALERATE AND DIAZINON IN SACRAMENTO SPLITTAIL (*POGONICHTHYS MACROLEPIDOTUS*) LARVAE.

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Four hundred, 7-day post hatching Sacramento splittail (Pogonichthys macrolepidotus) larvae were exposed to field water samples contaminated with esfenvalerate and diazinon using the USEPA standard static renewal method (EPA-600-4-91-002 7/1994) for acute toxicity testing. After the 96 hours exposure, mortality was recorded and fish were transferred and raised in clean well water at 18°C for three months. Cumulative mortality was determined, and individual fish were weighed, measured to determine condition index and sacrificed for biochemical and histopathological analyses. Concentrations of field samples for esfenvalerate and diazinon were verified analytically. There was no LD50 at the end of 96 hours exposure. However, significantly higher cumulative mortality and lower condition index were seen in exposed fish ($p<0.05$). Western blot analyses revealed significantly ($p<0.05$) elevated HSP 70 in exposed fish. Histopathologic analyses revealed severe cytoplasmic protein droplets and cell necroses in liver of fish exposed to field water contaminated with diazinon or esfenvalerate. Severe necrosis and pyknosis of the nerve cells were seen in fish exposed to field water contaminated with esfenvalerate. The results indicate a cause-effect relationship between sublethal concentrations of pesticides and biochemical and histologic changes in liver and brain. Splittail larvae, although surviving the 96 hours acute toxicity exposure, had significantly higher mortality rates and grew slower indicating that some of the fish were not able to recover from the toxic effects of the pesticides and these adverse health effects may be working to decrease splittail populations in the San Francisco Estuary. Finally, the study showed that the supplementation of sublethal toxicity endpoints to acute toxicity testing is important for investigating effects of contaminants in any aquatic ecosystem.

1101 SUBLETHAL TOXICITY OF THE PISCICIDE 3-TRIFLUOROMETHYL-4-NITROPHENOL IN AQUATIC MOLLUSCS AS MEASURED BY *IN VIVO* ^{31}P NMR SPECTROSCOPY.

M. R. Viant and R. S. Tjeerdema. *University of California, Environmental Toxicology, Davis, CA.*

Sensitive biochemical markers are needed to assess the actions of sublethal toxicant exposure on aquatic organisms. We have developed techniques utilizing *in vivo* ^{31}P NMR to characterize the responses of aquatic invertebrates to both chemical and natural stressors. NMR spectra were obtained using a Biospec-7T spectrometer and a customized probe, which houses a surface coil (2-turn, 3 cm diameter) placed adjacent to a Teflon animal chamber. The chamber receives a continuous flow of temperature-controlled, aerated seawater to which toxicants may be added. Our NMR methods allow the measurement of bioenergetically important phosphorus metabolites, including phosphoarginine (PA), inorganic phosphate (P_i) and ATP. Additionally, using saturation-transfer NMR techniques, we are able to measure arginine kinase (AK) reaction rates. Here we describe a comparative study on the sublethal effects of the piscicide, 3-trifluoromethyl-4-nitrophenol (TFM), on energy metabolism in the foot muscle of red abalone (*Haliotis rufescens*) and owl limpets (*Lottia gigantea*). Following 5-h acute exposures to 3 ppm (mg/L) TFM, abalone muscle became metabolically compromised, exhibiting both significantly elevated P_i and depressed PA levels. A corresponding increase in the AK reaction rate, in the forward direction, from 0.02 s^{-1} to 0.08 s^{-1} suggests that TFM compromises ATP production. This result is consistent with TFM acting as an uncoupler of oxidative phosphorylation. During a subsequent 5-h recovery period, in which the abalone received clean seawater, the metabolite levels and AK reaction rate returned to their pre-exposure values. The rapid clearance of the toxicant from the organism is consistent with the relatively low lipophilicity of TFM. Equivalent experiments on limpets produced similar results, although the effects were reduced in magnitude. Taken together, these data demonstrate that *in vivo* ^{31}P NMR is a viable technique for characterizing the sublethal effects of toxicants on whole organisms.

1102 SHORT-TERM ADAPTATION TO MERCURY RESISTANCE BY AQUATIC OLIGOCHAETES.

D. E. Vidal. *UC Berkeley, Ecological Engineering, Berkeley, CA.* Sponsor: A. Horne.

Aquatic invertebrates are widely used as indicators of environmental quality. The present research studies different mechanisms involved in the tolerance of aquatic oligochaete worms *Sparganophilus pearsei* and the relationships underlying their tolerance. The studied populations are located in mercury-contaminated fresh water reservoirs in the San Francisco Bay Area in Northern California. Mercury is highly toxic at any trophic level, bioaccumulates and biomagnifies through the food chain, and it is an integral part of human industry. LC50 studies established the organisms' tolerance levels for mercury. It was found that organisms from the most contaminated reservoir present a higher level of tolerance to the contaminant than the organisms from less contaminated reservoirs. Behavioral, biochemical and molecular mechanisms are studied to explore if this species uses autotomy (deliberate loss of caudal segments) to eliminate toxicants as well as proteins for detoxification. This study will provide a better understanding of how common pollutants induced the adaptation instead of the elimination of some aquatic organisms.

1103 RUBBER TIRE LEACHATE CAUSES TOXICITY IN DUCKWEED (*LEMNA MINOR*).

A. J. Draper. *Bucknell University, Chemistry, Lewisburg, PA.*

Tire rubber wears off automobile tires during regular use, and has been shown to result in particulate air pollution and in road dust and runoff water. When the larger tire particles run off roadways, they sink to the bottom of streams and leach chemicals into water. Aquatic organisms are exposed to these leached chemicals in the